REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the remarks presented herewith, which place the application into condition for allowance.

Status of the Claims and Formal Matters

Claims 1-3, 6-29, and 32-44 are currently pending in this application. Claims 16-21, 23, and 35-44 were previously withdrawn. Claims 4, 5, 30, and 31 were previously cancelled. Applicants assert the right to reclaim withdrawn or cancelled subject matter in co-pending applications.

Rejections under 35 U.S.C. §103(a)

Claims 1-3, 6-14, 22, 24, 25, 32, and 33 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths (U.S. Patent Application Publication No. 2002/0119459; "Griffiths") in view of Wangh et al (U.S. Patent Application Publication No. 2004/0053254; "Wangh") and further in view of Yu et al (U.S. Patent Application Publication No. 2001/0036632; "Yu"). The Office Action concedes that Griffiths does not teach methods of amplifying a nucleic acid wherein two populations of a first primer are used, one bound to a solid surface such as a bead, and the other in an amplification reaction solution. The Office Action also admits that Griffiths does not teach methods of amplifying one or more nucleic acids wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution and wherein substantially all of the molecules of the first primer species in the reaction solution are depleted. Further, the Office Action states that Griffiths fails to teach a method wherein a plurality of copies of the single stranded template nucleic acid are bound to the first population of the first primer species on the bead and wherein a bead-bound complementary strand is extended from the first primer species. Griffiths also does not teach microreactors having an average size of 50-250 μm in diameter.

Wangh allegedly teaches a method of performing non-symmetric PCR using one amplification primer that is present at a concentration at least five times greater than the other

primer such that the limiting primer can be used to exhaustion. According to the Office Action, Wangh allegedly teaches a method wherein one of the primers, the limiting one or the primer in excess, is fixed to a solid matrix such as a bead. Yu allegedly teaches a method for amplifying nucleic acids on a solid phase support wherein primers affixed to a solid support are hybridized to template nucleic acids, extended to form complementary products, and amplified using a solution phase reverse primer to form copies of the original template, which are subsequently annealed to additional immobilized primers for further amplification. Yu also allegedly teaches a method of asymmetric PCR using primers fixed to a solid support as well as in solution.

The Office Action contends that it would allegedly have been *prima facie* obvious to combine the method of Griffiths for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion on the surface of a bead with the method of Wangh for non-symmetric PCR using primers at unequal concentrations since this method is highly suited for amplifications that utilize small reaction volumes and very low copy numbers of target sequences. The Office Action further argues that it would allegedly have been *prima facie* obvious to attach a first population of a first primer to the solid surface to initiate primer extension on the template, including the use of fixed primers in asymmetric PCR as allegedly taught by Yu, since a second primer in a reaction solution and a second population of a first primer in the reaction solution can be used to perform amplification, wherein copies of the target can anneal back to the surface to initiate more rounds of amplification using the first primer.

Finally, the Office Action states that it would allegedly have been *prima facie* obvious to use emulsion droplets of larger sizes, such as in the range of 50 μm as used by Applicants or in the range of 10 μm as allegedly used by Griffiths, since these differences in emulsion droplet size would not be expected to greatly alter the conditions for amplification. The Office Action further contends that differences in the emulsion droplet size are considered routine optimization, and routine optimization is not considered inventive, absent evidence that the selection of droplet size was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

Applicants respectfully disagree with the contentions presented in the Office Action and traverse this rejection in view of the remarks presented herewith.

The present invention relates to, *inter alia*, a method of amplifying nucleic acids of interest using a novel and non-obvious asymmetric PCR procedure that utilizes three distinct primer species: (1) a first primer species that is immobilized on a solid support; (2) the same primer species as in (1), but which is in solution phase; and (3) a second primer species that is also in solution, but in vast molar excess to the solution phase primer species of (2). Steps (b) and (c) of instant Claim 1 defines a two-step asymmetric PCR method where solution phase amplification proceeds until the primer population of (2) is depleted. Thereafter, the PCR reaction occurs on the solid phase support as a result of hybridization to the immobilized solid phase primers of (1). Thus, the nucleic acid amplification reactions of step (c) are "driven" to the beads due to depletion of one of the solution phase primer species.

In the Response filed on September 19, 2007, Applicants previously argued that the combination of Griffiths and Wangh failed to describe the claimed invention. In response, the Office Action states at page 18, 1st paragraph, "[t]he Examiner agrees that, although Wangh teaches methods of primer depletion in an asymmetric amplification reaction on a bead, Griffiths and Wangh only teach amplification reactions using two populations of primers".

The Office Action now alleges that the addition of Yu cures the deficiencies of Wangh and Griffiths when used in combination. In particular, the Office Action at page 18 states "[i]t is obvious that the ***methods of Wangh using unequal concentrations of solution-based primers*** can be combined with ***the solid phase methods of Yu using one bound primer and one primer in solution*** to generate amplification products that are primarily bound to the solid support since the second primer in solution, as taught by Wangh, can be chosen as the more abundant primer species in solution" (emphasis added by Applicants).

Applicants respectfully assert that the combination of Wangh and Yu fail to describe all of the instant claim limitations, particularly the alleged connection of the species of primer present in both a population in the solution phase and a population on the solid phase. Importantly, there is no teaching or disclosure in any of the references that the solid phase primer species described by Yu is the same as the solution phase primer species described by either Yu or Wangh. In fact, Yu strongly suggests that the solution phase primer species are not identical to the solid phase primer species. See, for example, Yu at paragraph [0017]. Yu at paragraph [0047] also discloses that:

> "solid phase amplification of target polynucleotides from one biological sample is performed, wherein multiple groups of oligonucleotide primers are immobilized on a solid phase support. In a preferred embodiment, the primers within a group are identical in sequence and are selected or designed to be complementary to a defined sequence of one particular target nucleotide, capable of hybridizing to the target polynucleotide under appropriate conditions, and suitable as initial primers for nucleic acid synthesis (i.e., chain elongation or extension). Selected primers for each target polynucleotide are immobilized, as a group, onto a solid support at a discrete location."

Thus, Yu indicates that, as part of one group, primers immobilized to a solid support are identical in sequence, while the solution phase primers, as part of another group, contain a different sequence. There is no indication, teaching, or disclosure in Yu that the solid phase primer population and the solution phase population are the same primer species. Yu at paragraph [0050] teaches that the immobilized primer species anneals to the 5' end of the target nucleic acid, and paragraph [0051] provides that "[i]n practicing the present invention, the immobilized single primers can be used alone or, alternatively, in combination with primers in the reaction solution that are complementary to the sequence ***at the 3' end of the nascent immobilized strands***" (emphasis added), further supporting Applicants' argument that the two primer species disclosed by Yu are not the same.

The Office Action makes no specific argument that this claim recitation is taught by any combination of the cited references. Rather, the Office Action makes a statement that reinforces that there is no such specific teaching of this claim feature at page 18:

> "Copies initiated by the second primer will readily anneal to the bound first primer"

In other words the Office Action makes the specific point that the population of solution phase primer is different than the solid phase primer due to the complementarity of the "copies" to the solid phase primer. Importantly, the immobilized primer species of the presently claimed invention (i.e., the first primer species) is complementary to and binds *the target nucleic acid,* not the complementary copies as the Office Action asserts.

This is a very important element for the presently claimed invention because, as Applicants stated in the September 19th response, the asserted combination of Griffiths and Wangh fails to describe the use of primer depletion in a solution phase asymmetric amplification reaction *to promote binding of a selectively amplified template to a solid substrate*. In other words, without two distinct populations of the same primer species, one in solution and another immobilized on the solid phase the invention would fail to operate as intended. For example, as Applicants describe at page 6, lines 14-30 of the instant specification, the asymmetric amplification in the solution phase increases the concentrations of both the target strand and its complement from the "one single stranded template" until the limiting primer species in solution becomes depleted. Because the same primer species is present in the immobilized population, further rounds of amplification promote binding of the increased concentration of target molecules to those primers. As those of ordinary skill in the related art would appreciate, solution phase amplification is significantly more efficient than solid phase amplification. Thus, the initial rounds of solution phase amplification of the claimed method are important to efficiently increase the concentration of the target nucleic acid that is driven to bind to the solid phase, particularly when the limiting primer is depleted in the solution phase.

Applicants respectfully assert that Yu does not cure the deficiencies of the combination of Griffiths and Wangh, because contrary to the instant invention, Yu teaches that all of the described amplification occurs using an immobilized template producing an immobilized product. For example, Yu describes that in the "solid phase method" that includes solution phase primers, there is *no* solution phase amplification, rather Yu teaches that the first round of amplification uses *only* the immobilized primers to bind the target molecule and extend an immobilized complement (see paragraphs [0053] and [0056] of Yu). Subsequently, Yu teaches that the second round of amplification employs the solution phase primers to bind the immobilized complement product from the first round and extend an immobilized copy of the target, and that original target molecules perform another round of immobilized amplification similar to the first round. The results are immobilized double stranded molecules. For instance, Yu states in paragraph [0056]:

> "Meanwhile the original target templates hybridize to additional immobilized 5'-primer sites for further amplification. ***The results are double-stranded***

> ***amplification products on the immobilized primer sites***, with each strand being biotinylated, as shown in 2E". (emphasis added by Applicants)

In other words, Yu does not disclose solution phase amplification at all. In the second round of amplification, the solution phase primers bind to the *immobilized* product from the first round and extend an *immobilized* complement. Additionally, Yu provides no disclosure of another (third) population of primer species in the solution that is the same as the immobilized primer species that is used in solution phase amplification prior to solid phase amplification. In fact, Yu at paragraph [0048] discloses that the primer species are in vast molar excess relative to the concentration of the target nucleic acid, but provides no disclosure that one primer species is in excess of the other. This strongly suggests that all populations of primers described by Yu are in vast molar excess, which is clearly distinguishable from the presently claimed invention.

Applicants also respectfully assert that Wangh does not disclose the instantly claimed combination of solution phase asymmetric amplification with another population of the limiting primer species on a solid phase. Importantly, Wangh specifically describes a solution phase embodiment and a distinctly different solid phase embodiment, and it is clear from the disclosure that Wangh considered these two embodiments as distinct and should not be combined. In fact, where Wangh specifically considers immobilization of one of the primer species on a solid phase, there is an explicit teaching that the same primer species should *not* be present in solution. In other words, Wangh states that there should not be a second population of the immobilized primer species in the solution phase. For example, Wangh states in paragraph [0181]:

> "In another embodiment of the invention the LATE-PCR can be carried out under conditions in which one of the primers, most preferably the Excess Primer, is fixed to a solid matrix or surface such that each cycle of primer extension results in construction of an extended primer strand which remains attached to the solid surface, for example, a bead or the wall of the reaction chamber. It is anticipated that under these conditions $T_{m[0]}$ of ***the attached primer will be additionally dependent on the fact that the primer is not freely diffusible***, as well as by the packing density of the primer on the surface, by the volume, and by the geometry of the space in which the reaction takes place" (emphasis added by Applicants).

Thus, there is an express teaching by Wangh that if a primer species is immobilized on a solid phase, then that immobilized primer species should not also be in solution, thus preventing any solution phase amplification of the target of that primer.

In view of the foregoing remarks, Applicants respectfully contend that Griffiths, Wangh, and Yu, considered individually or in combination, fail to establish *prima facie* obviousness under §103(a), because the references do not teach or disclose all of the instant claim limitations, particularly three species of primers: two that are the same wherein one is immobilized in solid phase and the other in solution phase; and a third primer species that is present in solution in vast molar excess to the other solution phase primer. The deficiencies of the cited combination of references do not afford one of ordinary skill in the art with a reasonable expectation of success, especially in light of the disparate teachings of Wangh and Yu set forth above. For at least all of these reasons, the §103(a) rejection over Griffiths, Wangh, and Yu should be reconsidered and withdrawn.

Claims 15 and 26-29 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Wangh and Yu and further in view of Jurinke et al (U.S. Patent No. 6,303,309; "Jurinke"). The Office Action concedes that none of Griffiths, Wangh, or Yu teach a method for amplifying one or more nucleic acids wherein more than 10,000 or at least 1,000,000 amplification copies of each target nucleic acid molecule are bound to each bead. The Office Action also states that none of Griffiths, Wangh or Yu teach a method for amplifying one or more nucleic acids wherein the beads are Sepharose beads. Jurinke allegedly teaches a method of purification of biotin-labeled PCR products by complexing the products to a solid support containing a biotin-binding compound such as streptavidin immobilized on the surface, including agarose, sepharose, or magnetic beads. Jurinke also allegedly teaches immobilization of 100 pmol biotinylated oligodeoxynucleotide to 50 µl (~40 million) streptavidin-coated magnetic beads, which represents about 1 million molecules bound per bead.

The Office Action contends that it would allegedly have been *prima facie* obvious to combine the methods of Griffiths, Wangh, and Yu for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using non-symmetric PCR with that of Jurinke for purification of PCR products using solid supports such as magnetic or Sepharose beads, since the use of such beads allows further purification and extensive washing to remove all excessive

reaction components prior to final recovery of the final PCR product. Applicants respectfully traverse.

For reasons discussed herein, the combination of Griffiths, Wangh, and Yu do not teach each and every limitation of the instant claims and do not afford those ordinarily skilled in the art with a reasonable expectation of success, as required by §103(a). Jurinke fails to cure the deficiencies of Griffiths, Wangh, and Yu, because Jurinke merely relates to a method for dissociating biotin complexes comprising a biotin compound and a biotin-binding compound. The Jurinke method involves contacting the complex with an effective amount of an amine compound, which causes the complex to dissociate. Jurinke does not teach or disclose methods of amplifying nucleic acids on a solid phase support such as a bead, using two populations of a first primer that are present in each of a solution phase and in a solid phase, where the two populations are present in concentrations lower than the concentration of a second primer in solution phase. Because Jurinke is silent regarding methods of asymmetrically amplifying nucleic acids, Applicants respectfully contend that the combination of Griffiths, Wangh, Yu, and Jurinke do not teach or suggest all of the instant claim limitations and thus fail to establish *prima facie* obviousness under §103(a). Reconsideration and withdrawal of the §103(a) rejection over Griffiths in view of Wangh and Yu and further in view of Jurinke are respectfully requested.

Claim 34 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Wangh and Yu and further in view of Nakano et al. (J. Biotech. (2003) 102: 117-124; "Nakano"). According to the Office Action, Griffiths does not teach methods of amplifying a plurality of nucleic acid templates from 50-800 bp in length wherein two populations of a first primer are used, one bound to a solid surface such as a bead, and the other in an amplification reaction solution. Griffiths also does not teach methods of amplifying one or more nucleic acids wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution and wherein substantially all of the molecules of the first primer species in the reaction solution are depleted. The Office Action further concedes that Griffiths also does not teach a method wherein a plurality of copies of the single stranded template nucleic acid are bound to a first population of the first primer species on the bead, nor does Griffiths teach microreactors having an average size of 50 to 250 μm in diameter.

Wangh allegedly teaches performing non-symmetric PCR using one amplification primer that is present at a concentration at least five times greater than the other primer such that the limiting primer can be used to exhaustion. Wangh also allegedly teaches a method wherein one of the primers is fixed to a solid matrix such as a bead. Yu allegedly teaches a method for amplifying nucleic acids on a solid phase support wherein primers affixed to a solid support are hybridized to template nucleic acids, extended to form complementary products, and amplified using a solution phase reverse primer to form copies of the original template, which are subsequently annealed to additional immobilized primers for further amplification. Yu also allegedly teaches a method of asymmetric PCR using primers fixed to a solid support as well as in solution. Nakano allegedly teaches a method of PCR in water-in-oil emulsions using a plurality of templates of 528 and 512 bp in size.

The Office Action contends that it would allegedly have been *prima facie* obvious to combine the methods of Griffiths, Wangh, and Yu for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion for using non-symmetric PCR with that of Nakano, since Nakano allegedly teaches a method for amplifying multiple nucleic acids in water-in-oil emulsion that is allegedly easily adaptable to the method of Griffiths, Wangh, and Yu using a bead to bind amplification products in emulsion. Applicants traverse.

In view of the deficiencies discussed elsewhere in this Response, the combination of Griffiths, Wangh, and Yu do not result in the invention as claimed, nor does it result in an obvious variant of the instant invention. Nakano relates to single-molecule PCR using a water-in-oil emulsion, wherein limiting dilutions of the DNA template were used to create conditions where one molecule of template was amplified inside a droplet comprised of silicone oil, Triton X-100, and PCR buffer. Nakano does not remedy the defects of Griffiths, Wangh, and Yu, because Nakano does not teach, suggest, or disclose PCR that uses two primers in solution phase that are of unequal concentrations such that one strand of double-stranded template DNA is preferentially amplified over the other strand. Nakano is also silent regarding a method of amplifying a nucleic acid template on a bead, using two populations of a first primer species - one that is retained in the solution phase and one that is present on the surface of the bead, and a population of a second primer species that is present in solution phase in concentrations greater than either of the two populations of the first primer species. For at least all of these reasons,

Applicants respectfully contend that *prima facie* obviousness has not been established, because the combination of Griffiths, Wangh, Yu, and Nakano do not teach all of the instant claim limitations of Claim 34 (which depends from and encompasses the limitations of either of instant Claims 1 or 22). In the alternative, or additionally, the cited combination of Griffiths, Wangh, Yu, and Nakano fail to establish a reasonable expectation of success, particularly in view of the disparate teachings of Wangh and Yu regarding the primer populations as discussed herein.

For at least all of these reasons, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection over the combination of Griffiths, Wangh, Yu, and Nakano.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Applicants believe no additional fees are due with the filing of this Response. However, if any additional fees are required or if any funds are due, the USPTO is authorized to charge or credit Deposit Account Number: **50-0311**, Customer Number: **35437**, Reference Number: **21465-508 UTIL**.

Respectfully submitted,

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